Glycolysis compared in intact, permeabilized and sonicated L-929 cells

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The effects of incubation time and cell density on glycolytic rate were examined in suspensions of intact, permeabilized and sonicated L-929 cells. Sonicates exhibited strong dependence on cell density and a distinct lag in glycolytic rate, while intact cells showed no cell density dependence and linear glycolytic rates. Permeabilized cells exhibited linear glycolytic rates, but sometimes showed dependence on cell density. Rates of lactate production (nmol at 30 min/10^6 cells) were highest in sonicates and lowest in intact cells. These results are interpreted as support for the previously proposed hypothesis that enzymes of the glycolytic pathway are highly organized in intact L-929 cells.

Glycolysis; Cell sonicate; Permeabilized cell; Lactate; (L-929 cell)

1. INTRODUCTION

The assumption that enzymes released from cells during 'gentle' disruption of the plasma membrane were also freely diffusing in the intact cell has been seriously challenged by a number of researchers over the years, and particularly during the last decade. Indeed, the individual papers are so numerous that only selected recent reviews and books can be cited here [1-7]. These results also indicate that most of the enzymes of intermediary metabolism may be associated with each other, and/or with various ultrastructural elements in intact cells. The consequences of this 'organization' are far-reaching, not only in their obvious connections to metabolic regulation but also to structure-function relationships, notably in eukaryotic cell cytoplasm [8,9].

Our recent efforts have been directed toward ultrastructure, protein retention and release, and metabolism in dextran sulfate permeabilized (DSP) L-929 cells (L cells). This permeabilization procedure results in cells that retain about 85% of their protein and carry on a vigorous glycolysis, in spite of the fact that they allow the free passage of diffusing proteins of several hundred thousand daltons across their surface [10, 11]. In the present paper we compare glycolysis in DSP, intact and sonicated L cells. Because DS-permeabilization and sonication represent different degrees of disruption, we propose that the results can be used to extrapolate to the degree of organization of glycolysis in the intact L cell whose study is made difficult by the involvement of the plasma membrane.

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2. MATERIALS AND METHODS

L-929 cells (a fibroblast-like line from mice) from the American Type Culture Collection (Rockville, MD, USA) were grown to confluency in sealed flasks, and harvested by trypsin-treatment and washed as described in detail previously [11]. Intact cells were incubated in Dulbecco's phosphate-buffered saline (DBS) containing 10 mM glucose. Permeabilization with dextran sulfate (5×10^5 Da) followed, in general, the method of Kucera and Paulus [12] as fully described by us elsewhere [11,13]. DSP cells were incubated in Buffer K (150 mM sorbitol, 70 mM potassium gluconate, 5 mM $MgCl_2$, 5 mM NaH_2PO_4, 35 mM HEPES and 0.1 mM EGTA, pH 7.55) containing 5 mM glucose 6-phosphate (G6P), 2 mM ATP and 1 mM NAD^+ which lowers the pH to 7.4.

For complete disruption, L cells were sonicated, 1 ml at a time, in Buffer K at 4°C in a 12 mm diameter cylindrical chamber for 1 min at 40% power using the 4.8 mm microprobe on a Microson ultrasonic cell disruptor (Heat Systems-Ultrasonics, Inc., Farmingdale, NY). This was sufficient to reduce the cells to a suspension of subcellular particles. Sonicates were then incubated in Buffer K with 5 mM G6P, 2 mM ATP and 1 mM NAD^+. No difference in sonicate glycolysis was noted for preparations irradiated at 20% power for 30 s vs 40% for 60 s, even though less power and shorter time disrupted cells to a lesser extent. Glycolytic rate was somewhat lower at less than 20% power and 30 s duration.

Cell concentrations, trypan blue (TB) staining, and the extent of cell disruption in sonicates were determined with a hemacytometer (for details see [11]). Incubations took place in capped 12×75 mm glass tubes with 0.5 ml of a given concentration of cells (or cell equivalents for sonicates) quickly warmed to 37°C in a water bath and then continually mixed on a tilting orbital shaker for the appropriate length of time in a 37°C incubator. Glycolysis was stopped by addition of cold perchloric acid (PCA) to a final concentration of 6% and samples were kept on ice for at least 30 min. Lactate was measured in PCA supernatants using Sigma procedure no. 826-UV (Sigma Chemical Co., St. Louis, MO) as described previously [11]. Glycolytic rates were calculated per unit time as nanomoles lactate produced per 10^6 TB^- cells for intact populations, per 10^6 TB^+ cells for DSPs and per 10^6 cell equivalents for sonicates. Permeabilized (TB^+) cells require supplemental ATP and NAD^+ to glycolyze and so do not contribute to intact cell glycolysis, whereas intact (TB^-) cells cannot utilize external G6P and so do not contribute to DSP cell glycolysis. Sonicated suspensions were devoid of intact cells and so all were presumed to contribute their contents to sonicate glycolysis. Results

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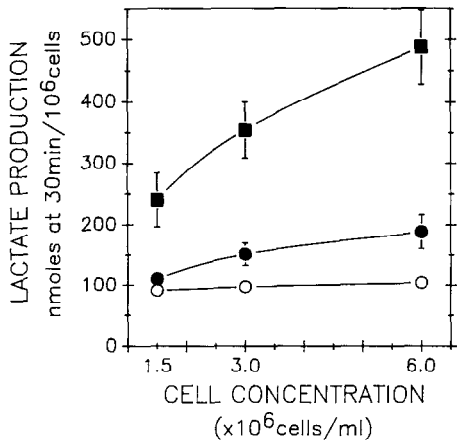

Fig.1. Effects of cell population density on the production of lactate by intact (○), permeabilized (●), and sonicated (■) L-929 cells after 30 min incubation. The bars are ± 1 SE, $n=4$. Error bars not visible are within the size of the symbol.

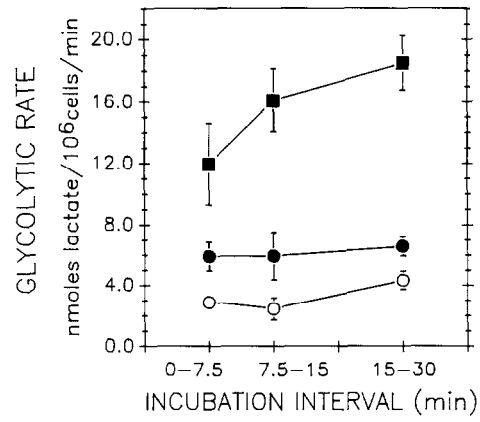

Fig.3. The kinetics of lactate production during different intervals of incubation for 6.0 × 10^6 cells/ml. The bars are ± 1 SE, n = 4. There are no significant differences between incubation intervals for intact (○) or DSP cells (●) whereas for sonicates (■) 0–7.5<15–30 min ($P<0.05$).

were analyzed using a two-way analysis of variance (ANOVA) followed by appropriate testing between means.

3. RESULTS

Fig. 1 compares the effects of cell concentration on glycolysis in intact cells, DSP-cells, and cell sonicates. In all cases the production of lactate in sonicates was strongly dependent on concentration. In addition, sonicates showed a considerable increase in lactate production over intact and DSP cells. As has been previously observed [11], DSP cells produced between 1.5 and 2 times more lactate than intact cells when originating from the same cell suspension.

DSP cells exhibited only a modest and not significant degree of cell concentration dependence. Although DSP preparations usually show no cell concentration dependence, occasionally a significant effect is observed. This variability has been noted in previous work [11] and again here as illustrated in fig. 2. This graph shows the concentration dependence exhibited by four different preparations of DSP cells after 30 min of incubation and is representative of the range and frequency of concentration dependence we have observed for DSP cells.

Fig. 3 illustrates the kinetics of lactate production in sonicated, permeabilized and intact L cells. Each point represents the rate of lactate production per min for the given incubation interval; therefore, no change between intervals indicates a linear rate of lactate production. Glycolytic rates were linear in intact and permeabilized cells, with no lag in lactate production in both cases. Sonicates clearly exhibited nonlinear glycolytic rates with a significant lag for the first 7.5 min. Fig. 4 shows an experiment which clearly illustrates these kinetic characteristic as well as the interaction between time and cell concentration on glycolytic rate.

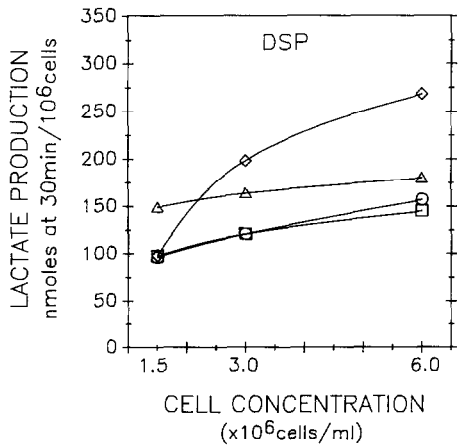

Fig.2. Effects of cell density on lactate production for four separate preparations of permeabilized cells (different symbols).

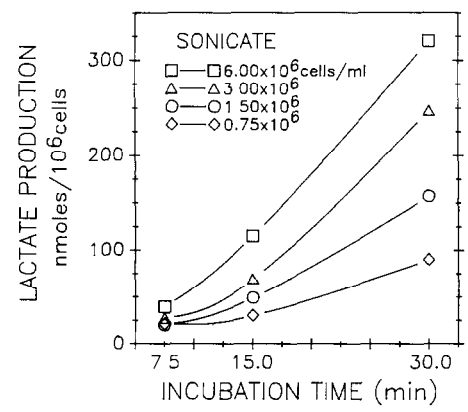

Fig.4. Lactate production in sonicates of L cells, as a function of incubation time, for different cell-equivalent concentrations.

4. DISCUSSION

Glycolysis in intact L cells and their sonicates exhibits a high degree of repeatability in terms of the effects of cell concentration and incubation time: intact cells show density independence and relatively linear rates, whereas glycolysis in sonicates is non-linear, and strongly density-dependent. The results with sonicates are precisely those expected for the participation of individual, freely diffusing enzymes and intermediates which must transit between enzymes. It is most likely that the initial lag results from the necessity to build up intermediate concentrations to levels required for steady state glycolysis. The dependence on cell density would, in that case, be well understood since these concentrations can be achieved more rapidly at high cell densities.

In contrast, DSP cells exhibit more variability and appear to be intermediate compared to the behavior of intact cells and sonicates, depending on the particular DSP preparation. We believe this variability is due to different degrees of disruption caused by DS-treatment, both with regard to the extent of plasma membrane removal and cytoplasmic disruption, for which we have direct ultrastructural evidence [11], and the degree to which enzyme–enzyme and enzyme–cytomatrix associations are also disturbed, a possibility we raise in the present paper. In support of that proposal are differences observed in the amount of lactate produced by equivalent amounts of cells: sonicates >> DSP cells > intact cells. That result is particularly surprising since in sonicates the enzyme concentration (per unit volume of total reaction mixture) is much lower than in equivalent numbers of intact cells (per unit volume total cell water, at the very least) or in DSP cells, which we have shown retain their glycolytic enzymes [11]. We suppose that glycolytic enzymes in sonicates have lost the structural and functional regulators that control the rate of this pathway in intact cells. Likewise, we suggest that the glycolytic rates of DSP cells, being intermediate in rate, reflect an intermediate degree of disruption between the intact cell and the totally demolished one (sonicate).

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